THE DEVELOPMENT OF THE SEED IN THE PONTEDERIACEAE

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(WITH PLATE XXIII)

A chance section of the young seed of Pontederia made several years ago presented a peculiar appearance in the antipodal end of the endosperm that led me to make an investigation of seed development in this family. I have been able to study *Pontederia cordata* and *Heteranthera limosum* in some detail, and *Eichhornia crassipes* to a less extent. Collections of Pontederia have been made in South Carolina and New York for several years. All my Heteranthera and Eichhornia material was collected in Jamaica during August 1900.

Examination of the literature of this family shows but one title dealing with its embryology. In 1898 Smith (**14**) published a rather full account of seed development in Eichhornia and Pontederia, and gave one figure of the embryo sac of Heteranthera. Oddly enough, he completely overlooked the interesting peculiarity that appears in the endosperm of all three of the above genera, but otherwise, where parallel, my work, with a few exceptions, agrees with his. As Smith did not attempt to give the development of Heteranthera, I have made a careful study of its ovular growth as a comparison with his work on Eichhornia and Pontederia. In the last two genera I have confined myself to the origin and development of the endosperm, which has the same peculiarity in all three types. Hofmeister (**9**) devotes a few lines to the development of Pontederia, but does not mention two sorts of endosperm.

HETERANTHERA

The megaspore mother cell of Heteranthera is separated from the epidermis by a single tapetal layer, as is commonly the case in monocotyledons (*fig. 1*), and it reaches a very conspicuous size before its first division (*fig. 2*). The spindle of this division lies near the distal end of the cell, and cuts off a small upper and a large lower cell (*fig. 3*). Each of these now divides (*fig. 4*), and the result is a large, lower,

viable megaspore, with three small potential ones above. The two upper spores may either lie side by side (*fig. 5*), or one over the other (*fig. 5a*), agreeing in this respect with Eichhornia (SMITH). The division line between the upper two cells is very obscure, and as the cells soon show a sign of disorganization the cell wall separating them soon becomes invisible. In Eichhornia, SMITH describes two methods of megaspore formation from the mother cell; one as I have just described for Heteranthera, and another in which "the elongation of the ovule is accompanied by an elongation of the primary sporogenous cell without division of the latter. . . . The subsequent divisions in the elongated sporogenous cell take place in quick succession." He further says, "From a study of a large number of cases, similar to figs. 14 and 15, I have been led to believe that the four mother cells when formed in this way are seldom, if ever, separated by walls." I have noticed nothing in Heteranthera to indicate that there are two methods of megaspore formation. Walls are always formed in both first and second divisions, and the divisions occur in all cases as I have described.

Almost as soon as formed, the large lower megaspore commences to elongate and encroach on the ones above it, and after destroying them it pushes on through the tapetum and reaches the inner edge of the epidermis (*fig. 6*). Division of the nucleus now takes place and the embryo sac is soon organized (*figs. 6* and *7*). The structure of the sac is perfectly normal at this time; the synergids are rather sharply pointed and are decidedly denser than the egg; the antipodals are quite distinct, though small. SMITH states that in both Pontederia and Eichhornia the antipodals are ephemeral, disappearing early, but I have found this not to be the case in either Heteranthera or Pontederia. The lower end of the sac where the antipodals rest develops scarcely at all in the subsequent growth of the ovule, and remains as a little projecting pocket from an apparent basal invagination (*fig. 9*). In *fig. 8* is represented a sac at about the time of fertilization. The definitive endosperm nucleus is very large and conspicuous, being in fact as large as the egg or synergids.

At the stage represented in *fig. 9* a cell wall is present, cutting off the lower end of the sac into a separate chamber. In this chamber are a number of endosperm cells in addition to the three antipodals

which still occupy their little pocket. The origin of this lower tissue has not been determined in all stages for Heteranthera, but from its development in Pontederia there is no doubt that it arises from the division of the lower of the two nuclei produced by the first division of the definitive endosperm nucleus. A later stage of this lower endosperm is shown in *fig. 10*. The section shows five cells below the diaphragm in addition to the antipodals. It will be seen that the cells below are larger than those above the partition. As this tissue has been studied most carefully in Pontederia, I will not go into detail here in regard to it, as it seems the same in both cases.

The structure of the nearly mature seed of Heteranthera is of sufficient interest to warrant some description. The axial embryo extends nearly the whole length of the seed, and is surrounded by a large-celled endosperm, which, except in its immediate neighborhood, is densely packed with starch. In *figs. 11* and *12* are shown longitudinal and cross-sections of the seed coats and superficial endosperm. The nucellus is entirely destroyed except for the scarcely recognizable micropylar cap, and the aleuron layer of the endosperm lies in immediate contact with a heavy cellulose membrane secreted by the inner integument. The inner integument (**11**) is considerably crushed and its cells are so densely filled with deeply staining material that their outlines are not recognizable except in unusually favorable conditions. Beyond the inner integument is a still heavier cellulose membrane, represented by a white line in the figures. This membrane seems to be secreted by the cells of the outer integument, which remains permanently two layers of cells in thickness. The outer layer changes very little in appearance during the development of the seed, remaining thin walled and boxlike, with the long axis running parallel to the longitudinal axis of the seed. The cells of the inner layer, on the contrary, undergo great changes. They become much elongated, perpendicular to the long axis of the seed, and flattened in the contrary direction. The partitions parallel to the long axis of the cells are much higher at their ends than in the center, and their outer edges dip inward in a regular curve (*fig. 12*). As the cells of the outer layer follow this curve, the result is that the surface of the seed becomes channelled and ridged in a longitudinal direction. A longitudinal section of the seed taken near the top of a ridge would

appear as in *fig. 11*. Taken in a channel it would be the same except for the height of the inner layer of the outer integument. At the time represented in the figures, which is shortly before the full maturity of the seed, these large cells are found to be multinucleate, with nuclei and protoplasm in apparently normal activity. In *fig. 12* five nuclei are shown in a single section; they seem to have been formed by ordinary mitotic division. Note that, contrary to what might be expected, they are on the outer side, where the cell walls are thin.

PONTEDERIA

As SMITH has followed the early development of the embryo sac in Pontederia, I have taken up its history at a point just preceding the formation of the endosperm. In *fig. 13* is shown a sac just before the division of the definitive endosperm nucleus. This nucleus lies very low, just above the antipodal pocket, and in this position it remains until its division. As a result of the first division, a cross-wall appears, cutting the sac into a large upper chamber and small lower one (*fig. 14*). The endosperm nuclei in each chamber now divide repeatedly and the protoplasm increases rapidly in amount. As already mentioned, the lower tip of the sac does not increase in size, and as the part above grows outward, and develops downward at the edges, the antipodal pocket comes finally to hang from the center of a considerable depression (*fig. 17*). The antipodals never divide, but they are not ephemeral, as described by SMITH. They are easily visible after cell walls have appeared in the endosperm.

In the upper chamber of the sac the development of the endosperm presents no great peculiarities; cell formation begins only after the sac has increased in size many times and the embryo has become conspicuous. In the lower chamber the protoplasm increases rapidly in extent and density (*fig. 17*), and the nuclei multiply by mitotic division until their number becomes quite large, fifteen appearing in the single section shown in *fig. 17*. Soon after cell walls begin to appear in the upper chamber, they are also formed in the lower, but in a much more irregular way. From the first the cells cut out of the basal endosperm are often multinucleate, while those above are uninucleate, as usual, when first formed. Another point of difference is that the cell walls in the antipodal endosperm soon become

much thicker than in the tissue above (*fig. 18*). As the growth proceeds their nuclei become very irregular, stain deeply, and show a tendency to fuse together. The protoplasm also becomes much denser than that of the upper endosperm (*fig. 18*). In *fig. 19* are represented several cells of the antipodal endosperm more highly magnified; they are from a stage slightly later than represented in *fig. 18*.

As the maturity of the seed approaches, and the upper endosperm begins to be filled with starch, the cell contents of the lower endosperm show signs of disintegration. The nuclei become deeply staining and irregular masses, and the protoplasm loses its distinctive structure and begins to disappear. No starch was found in these cells at any time, and at the maturity of the seed they appear, like the cells immediately above them, empty and somewhat crushed at the tip of the embryo.

The number of cells in the basal endosperm may become quite large. I have counted as many as forty-five in a single section, and there must have been in that case at least two hundred.

The seed coats of Pontederia are very much like those of Heteranthera, except that the outer integument is three cell-layers thick, with the cells of the two outer layers thin and boxlike, and those of the inner of the same height throughout, giving a smooth, instead of ridged, surface to the seed.

EICHHORNIA

My material of Eichhornia was not sufficient to follow the development of the basal endosperm, but its presence is shown in my sections at several stages. It seems to be similar in every way to that of Pontederia and Heteranthera. The structure of the seed coats is exactly as in Heteranthera. The same figures would do for both.

CONCLUSION

In looking over the literature of the subject I find but a single case where a tissue similar to this has been described, and there its origin has not been determined in detail. BILLINGS (1) has found that in *Tillandsia usneoides* there is formed in the lower part of the embryo sac, just above the antipodals, a tissue which in structure and position much resembles that of the Pontederiaceae. He does not

give the exact origin of this tissue, but says: "the nuclei resulting from the first divisions of the endosperm nucleus take position at either end of the sac, leaving, however, a few to form a thin parietal layer between. At the antipodal end, cell formation with walls begins at once, and a number of large cells form a tissue which stands out conspicuously in the cavity of the sac, which otherwise contains only a few free endosperm nuclei." Tillandsia is considered a near relative of the Pontederiaceae, and it does not seem improbable that the origin of this tissue is the same in both cases. A difference to be noted is that in Tillandsia the basal endosperm tissue, according to BILLINGS, is cellular from the first.

The appearance of the cross-walls at the lower end of the sac at the first division of the endosperm nucleus will at once recall a similar condition in Sagittaria (SCHAFFNER **13**), *Potamogeton pauciflorus* (WIEGAND **16**), and Ruppia (MURBECK **12**). In these cases, however, the nucleus in the lower chamber does not divide to form a tissue, as in the Pontederiaceae.[1]

In *Potamogeton foliosus*, according to WIEGAND (**17**), the young embryo sac has almost the same appearance as in Pontederia; but this structure is supposed to have come about in a different way. The wall that cuts off the lower end of the sac is derived from the division of one of the first four nuclei of the sac, its sister nucleus forming the three antipodals which occupy the little pocket below. The author thus regards the large nucleus which occupies the lower end of the sac as an antipodal, as it is the sister nucleus of the lower polar. No upper polar nucleus is supposed to be formed and the endosperm develops from the lower polar. This whole procedure is so peculiar that it seems open to some doubt until confirmed.

In *Potamogeton natans*, HOLFERTY (**10**) finds the embryo sac in general quite normal, but in two or three cases a large nucleus was discovered in the lower end of the sac, cut off above by a wall. He thinks it may have originated in the same way as I have described for Pontederia.

In *Castalia odorata*, *C. ampla*, *C. pubescens*, *Nymphaea advena*, *Brasenia purpurea*, and *Cabomba piauhiensis*, COOK (**5, 6**) has found that an antipodal haustorium, cut off from the rest of the sac by a

[1] In Sagittaria, according to SCHAFFNER, it may divide once or twice.

wall, contains a large nucleus which has the same origin as in Pontederia. This is also just what is found in the case of *Saururus cernuus* (JOHNSON **11**), but in none of these cases does the lower endosperm nucleus divide.[2]

In Limnocharis, according to HALL (**8**), the lower of the two nuclei formed by the division of the megaspore nucleus passes to the lower end of the sac, but does not divide to form antipodals and a lower polar nucleus. The upper polar nucleus, formed in the usual way, sinks to the lower end of the sac, and there divides without fusion (there being no lower polar). A cell wall resulting from this division cuts the sac in two. The nucleus left in the upper cell divides to form the endosperm, but the lower does not divide.

In both Naias and Zannichellia, CAMPBELL (**3**) has described a large nucleus near the antipodal end of the sac, which increases greatly in size, but does not divide. He did not determine the origin of this nucleus, but offers two suggestions, either that the large lower nucleus is the lower polar, or that it is the product of the first division of the definitive endosperm nucleus. The latter explanation seems most probable.

In *Elodea canadensis*, WYLIE (**15**) finds that there is generally present a large extra nucleus in the antipodal end of the sac. He says: "The sudden appearance of the extra nucleus in the antipodal group, when one has in mind the behavior of the polars, might suggest that these nuclei do not always fuse, and that one of them passes down to the lower end of the embryo sac and joins those in the antipodal pouch. The general evidence, however, is against this view. The irregular number of nuclei displayed here and the general arrangement of the cytoplasm about them would indicate that any increase in number has come from division among the antipodals, an activity in this region that often results from fertilization. In all embryo sacs studied at earlier stages the lower polar had passed out of the tip, and its return to the antipodal group seems improbable." Is it not more probable that this nucleus is formed as in Pontederia?

[2] COOK mentions a single division occurring once in *Nymphaea odorata*.

This discussion might be indefinitely extended by referring to the numerous cases of chambered sacs and other peculiarities of the endosperm of dicotyledons, but homologies are so improbable in such details that it is not necessary to cite them further. A good exposition of this subject may be found in COULTER and CHAMBERLAIN'S *Morphology of the angiosperms*.

An increase in the number of antipodals by division of the original three has been reported in a number of families, but, so far as I can discover, an endosperm tissue of the origin and character here described for the Pontederiaceae has not been before described. Its closest approach is probably to be found in Tillandsia.

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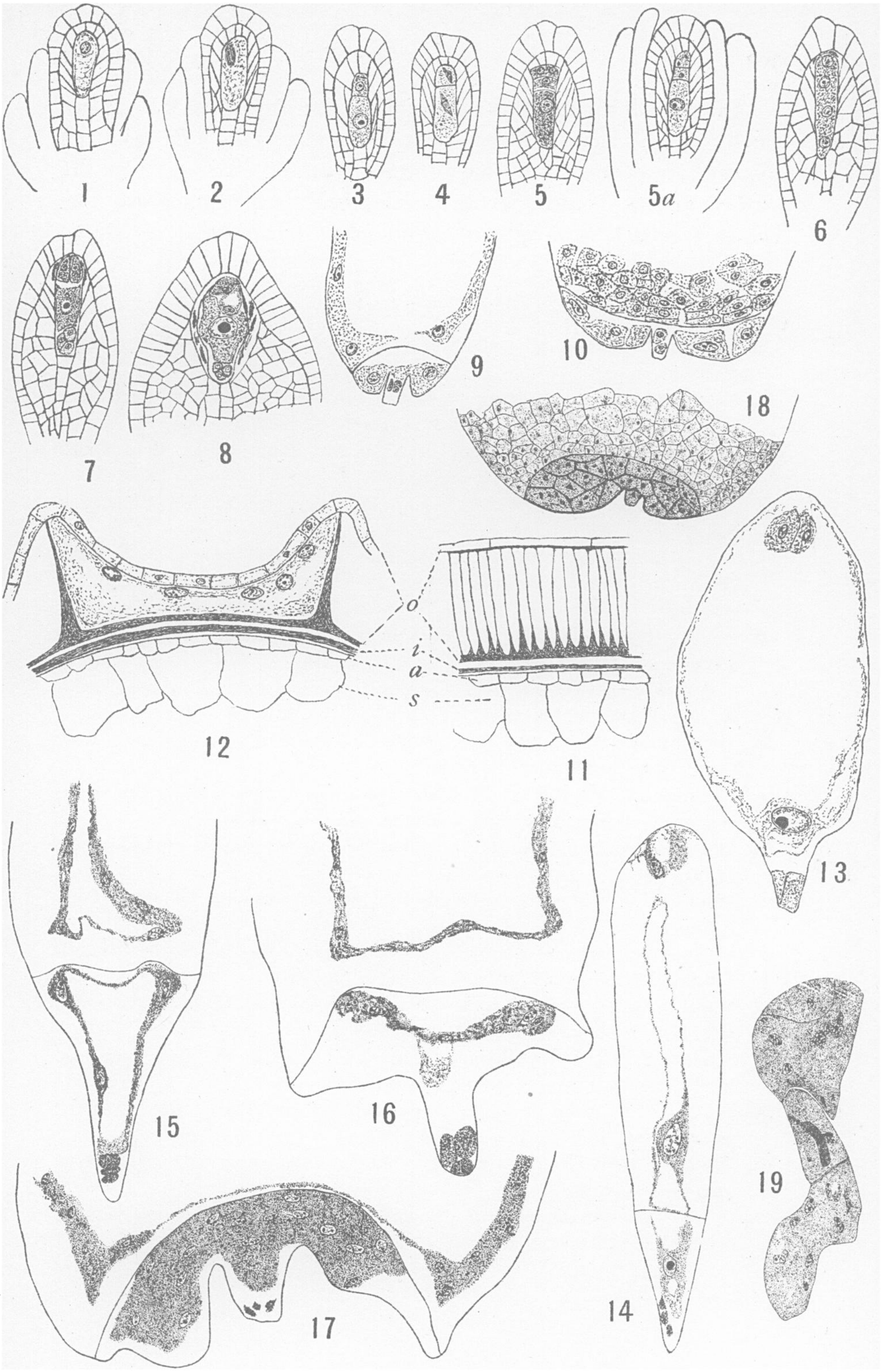

COKER on PONTEDERIACEAE

EXPLANATION OF PLATE XXIII

The magnifications given are for original drawings, which have been reduced one-half in reproduction.

Figs. 1–12. Heteranthera limosum

FIG. 1. Young ovule, showing megaspore mother cell. ×675.

FIG. 2. Mother cell in division. ×675.

FIG. 3. Result of first division of mother cell. ×675.

FIG. 4. The two daughter cells dividing again. ×675.

FIG. 5. Shows four megaspores; the two upper side by side. ×675.

FIG. 5a. Four megaspores in a row. ×675.

FIG. 6. Four-celled embryo sac. ×675.

FIG. 7. Eight-celled embryo sac. ×660.

FIG. 8. Embryo sac ready for fertilization. ×675.

FIG. 9. Lower half of older embryo sac showing antipodals and basal endosperm. ×450.

FIG. 10. The same after formation of cell walls. ×450.

FIG. 11. Longitudinal section of seed coats and superficial endosperm. *a*, aleuron layer; *i*, inner integument; *o*, outer integument; *s*, starchy endosperm. ×780.

FIG. 12. Cross-section of same. Letters as in *fig. 11*.

Figs. 13–19. Pontederia cordata

FIG. 13. Embryo sac just before division of definitive endosperm nucleus. ×670.

FIG. 14. Embryo sac just after said division. ×670.

FIG. 15. Later stage showing antipodals, basal endosperm, and upper endosperm. ×670.

FIG. 16. Still later stage of same. ×670.

FIG. 17. Just before beginning of cell-wall formation in endosperm. ×670.

FIG. 18. Showing both kinds of endosperm after formation of cell walls. ×150.

FIG. 19. Several cells of basal endosperm at about the same stage as *fig. 18*. ×670.